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Efficacy of *Pseudomonas* spp. NA-4 against *Meloidogyne incognita*

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In concern with our present studies, this paper basically put lights on the efficacy of biocontrol agent, *Pseudomonas* strain NA-4 for the management of *Meloidogyne incognita* in tomato. Four plants were kept under observation. The one which remain untreated shows more vigorous growth while our main plant which is inoculated with both nematode and bacteria shows that bacteria has shown some control against nematode activity while the plants which are inoculated with nematode alone shows reduction in height and other parameters. Bacterial inoculated plant grow normally even because of its growth promoting nature due to some dramatic changes in climatic conditions and other external or internal factors.
Keywords: Control, biological agent, plant growth, root knot nematode, Solanaceae.

INTRODUCTION

Lycopersicon esculentum, the tomato is considered the most important fruit vegetable in world, which lies in the nightshade family, solanaceae (Isah *et al.*, 2014). It is one of the many vegetables, which are being eaten widely worldwide. In Pakistan, it is used as both fruit and vegetable, but its consumption as vegetable is more. In Pakistan, total field area under this crop was 27.9 to 50 during 2009-10 (Khokha, 2013).

Area regarding to the cultivation of tomato crop has been increased up to 62000 Ha and the outcome is almost 600 tons annually, which is relatively low as compared to the other tomato producing countries in the entire world (Atta ur Rehman Khan, 2017). Therefore, relatively more attention is given to its cultivation and area because it tends to give high satisfactory outcomes in very short duration to the grower (Khokha, 2013). Tomato is full of nutrition that have high amount of all the essential sugars, vitamins, proteins and minerals. It is served as salads or as in processed form like soup and sauces (Ali *et al.*, 2020).

Many diseases tend to effect tomato by many economically devastating pathogens like fungi, bacteria and nematode. Out of these detrimental pathogens, major yield losses which deteriorate both quantity and quality of the tomato crop is by plant pathogenic pest, root knot nematode. *Meloidogyne incognita*, a root knot nematode which effects tomato crop severely by retard the growth of tomato plant. Among major plant causing diseases biotic agents, root knot nematode is on top of the list while in the world it rank first among the ten main and economically devastating genera of nematode, which cause plant diseases exclusively (Mukhtar *et al.*, 2017). Root knot nematode have the ability to be combine with other disease causing organism and tend to behave as complex disease implication as well as causing severe wilt problems in crops. In Pakistan, *Meloidogyne incognita* is spreading very quickly among all the vegetables growing areas which is causing very low yield and ultimately affect the growers and the consumers severely. *Meloidogyne incognita* is spread around the world in the percentage 47% while Pakistan have about 52% distribution of this specie with an estimated effect on tomato is in terms of 24% to 38% loss in yield (Mukhtar, 2018).

The main reason of the reduction in yield in tomato crop is successfully establishment of pathogen and buildup of its inoculums as well as continuous cropping practices in an area. Combating these detrimental nematodes has become very obvious now. *Meloidogyne Incognita* can be manage to some level by practicing physical control such as rotating crop, steaming the soil, fallowing of land, flooding of land and using disease-free propagator materials but its usage to manage these pest has been because of high cost, difficult to apply, less efficiency and not much economical (Muhammad Arshad Hussain, 2016).

The use of nematicides to inhibit and restrict nematode growth has its demerits also. Generally, nematicides and

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growing resistant varieties are considered such methods, which can control diseases cause by root knot nematodes.Using nematicides for controlling root knot nematode has more demerits rather merits because using chemicals to kill nematodes requires very high cost, effect crop and human badly, availability is difficult, building of resistance in nematodes by evolution, hazardous effects on environment, capability to build toxicity by residues and harmful effects on beneficial soil microorganisms.

However, using biological control for the management of root knot nematode proves to be effective and ecofriendly as compare to all the above-mentioned methods (Mukhtar *et al.,* 2017). Biological control for the nematode growth can be reduced to great extent and has many benefits with relatively low or no detrimental effects (Mukhtar, 2018). It is preferable above all the methods to reduce or manage root knot nematode and this method can be applied with all the other practices, which cumulatively aid for the integrated nematode management program (HOESTRA, 1979). Biological control is best method because it tend to reduce nematode population effectively by direct suppressing reproduction rate and its very ecofriendly, cost effective, provides long term benefit and have no effects on human and the crop (Mukhtar, 2018).

Keeping in consideration of above study we used bacteria as biocontrol agent for the management of *Meloidogyne incognita* which was our main objective. Beside using Pseudomonas strains NA-4 as plant growth promoting rhizobacter for the tomato plant and checking its efficacy on it, this biocontrol agent can be used effectively against *Meloidogyne incognita* with the main objective to minimize disease incidence rate cause by this nematode and to provide fresh and healthy tomatoes both quality and quantity wise for the successful consumption and utility of final consumers.

MATERIALS AND METHODS

The materials and methods involved in our project are;

***Raising of nursery*:** Soil were prepared for the sowing of tomato seed variety Roma. Nursery soil contained normal soil and sand relatively 60:40 respectively. Filled the pots with the prepared proportion of the soil for raising nursery. 10-12 number of seeds were sowed per pot. Covered the pots with the polythene sheet to provide favorable environment for the germination. The pots were irrigated at the 4 days interval regularly with the sprayer. The seedlings were germinated within 7 days. After 20 days the seedlings were ready to transplant.

***Transplanting*:** Tomato nursery was prepared after 20 days of the sowing. Before a day of transplanting the pots were irrigated so that, the plants can easily uproot or transplant. At the time of transplanting, a seedling was containing 3-4 leaves. Transplanting were done at the evening time so that seedlings can be easily established. After transplanting pots were covered with the polythene sheet.

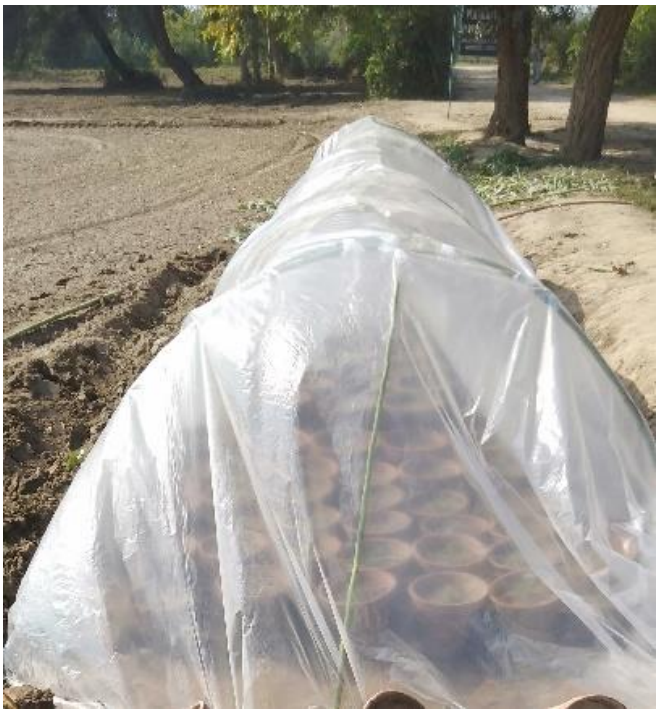


Figure 1. Cultivation of Tomato under greenhouse.

Microbial strains

***Extraction of the Meloidogyne spp. from soil*:** Nematode (Meloidogyne sp) were isolated from the soil by the Hemming Tray Method. Two plastic trays were taken and lined with the 1mm and 50mm plastic meshes and were supported by the tissue paper.50g 0f hand crumpled soil sample were taken and spread thoroughly on the tissue paper. The required quantity of the tap water was taken in the lower tray so that it can slightly touch the tissue in the upper tray. After 2 days the tissue paper was removed with the meshes and lifted soil particle. A small amount of water (approximately 100mi) were used to rinse the tray. The extracted was poured into a beaker and allowed for sediment for 12 hours. After this it were collected into a tube. Suspension were taken on the glass slide to examine the number of nematodes under the stereomicroscope. The Doncaster dish were used to count the total number of nematodes.

Figure 2. Extraction of Nematode (Nematodes ware extracted from the effected soil by using Hemming Tray Method).

Mass culture of the pseudomonas sp. NA-4:
The further multiplication of the culture was done by streaking on Nutrient Agar (NA).

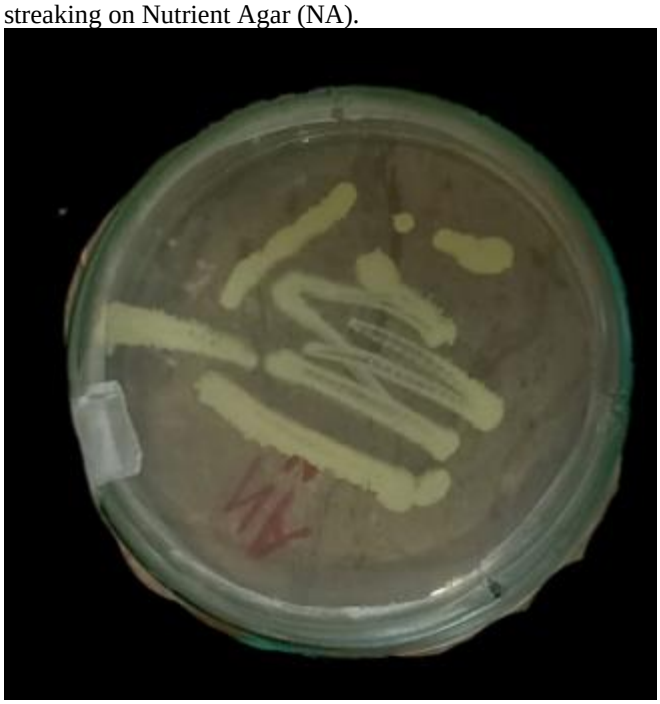


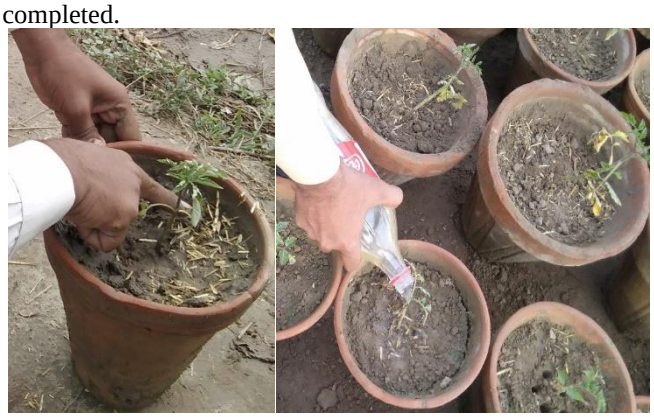
Figure 3. Bacterial culture (The culture of *pseudomonas* spp. NA-4 used in this experiment)

INOCULATION

***Inoculation of pseudomonas sp NA-4*:** After 10 days when the transplanted seedlings fully established, they were inoculated with the *pseudomonas* sp NA-4.

The culture of the pseudomonas was mixed with the wheat straw. The roots of the transplanted plants were exposed so that the culture of pseudomonas can be inoculated around the roots easily. After the inoculation the roots were covered with the culture with the wheat straw and small amount of soil.

***Inoculation of nematode*:** The 5ml suspension of the nematode was taken in a beaker for a plant which contained 2000 number of nematodes. The small holes were made near the root zone of a plant. The suspension of the nematode was poured into the holes which were made for the inoculation of nematode. In this way the inoculation of the nematode completed.



A **B**

Figure 4. (A)(Left) Inoculation of Bacteria (The *pseudomonas* spp. NA-4 was inoculated with the wheat straw). (B)(Right) Inoculation of Nematode (The Inoculation of Nematode suspension in the roots of tomato plants).

In our Biocontrol process four treatments were applied in which Bacterial treatment were used for promoting enormous growth of plant, Nematode treatment is used to infect plant and check its effect,Bacteria+Nematode treatment were used as biocontrol in which bacteria were used as biocontrol agent to control the growth of nematode and in the last Control (untreated) plant were used which grow best without any effect.

***Data parameters*:** The results of scientific report includes following data parameters

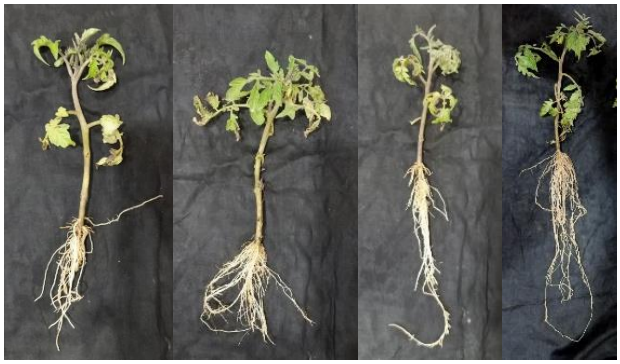
- Plant height
- Plant weight
- No. of flowers
- No. of leaves
- No. of galls

***Plant height*:** The height of the healthy plant(65cm) is more as compared to nematode(42cm), bacterial(38cm) and nematode+ bacterial average(25.33cm) treated plants

Table 1. Calculated Parameters of the Experimental Tomato plants .

Treatments	Height (cm)	Shoot height (cm)	Root Height (cm)	Total weight (g)	Shoot weight (g)	Root weight (g)	No. of flowers	No. of leaves
Bac+ Nematode 1	28.00	14.00	15.00	6.71	4.41	2.30	0.00	19.00
Bac + Nematode 2	24.00	13.00	11.00	3.65	2.27	1.30	0.00	15.00
Bac + Nematode 3	24.00	12.00	12.00	4.50	3.10	1.41	1.00	16.00
Average	25.33	13.00	12.66	4.95	3.26	1.67	0.33	16.66
Healthy	65.00	26.00	39.00	17.67	11.62	6.05	4.00	35.00
Nematodes	42.00	17.00	25.00	9.18	6.94	2.24	4.00	41.00
Bacteria	38.00	12.00	26.00	6.58	4.22	2.36	0.00	40.00

***Reasons*:** The reason is that healthy plant is provided with optimum conditions, proper nutrition, proper availability of water and no treatments are applied on this plant.
***Plant weight*:** The weight of healthy plant is 17.67g which is more than others because of presence of extensive roots and shoots and height of healthy plant is also higher than others. As compared to healthy plant, nematode treated plant weight is 6.58g which is less than bacteria treated plant whose weight is 9.18g, bacteria nematode treated plant weight is 4.957g
***No. of flowers*:** 4 flowers are present in case of healthy plants and in nematode treated plants and no flowers shown in case of bacteria treated plant and in (bacteria +nematode) treated plants.
***No. of leaves*:** 35 leaves are present in healthy plant ,41 leaves in nematode infected plant ,40 leaves in bacterial plant and average of 16.67 leaves in (bacteria+nematode) treated plant
***No. of Galls*:** No galls present in case of nematode treated plant because of some external and internal factors affect.



A B C D

Figure 5. (A) Nematode + Bacteria (B)Bacterial (C)Nematode control (D) Healthy.

DISCUSSION

By observing results, healthy plant showed the more vigorous growth as compare to bacterial, nematode and bacteria plus nematode inoculated plants. The reason behind its more profuse growth is that tomato plants which remain untreated have the ability to grow well by maintaining proper conditions in fields. Whereas those plants which have been treated either with nematode, bacteria or nematode plus bacteria inoculated. Healthy plant grow well because it has maximum opportunity to get all the optimum conditions which could aid for its proper growth, however by adding bacteria in one of the plant which is *pseudomonas* strain NA-4, a growth promoting rhizobacter which has tendency to enhance growth in plant didn't work quite efficient.
The plant inoculated with bacteria *Pseudomonas* could promote plant growth. Because of its nature to enhance plant growth in tomato.The tomato plant showed height but not more than healthy one because of some external and internal bacterial inhibitory factors. In our project bacteria showed some promotion in growth. The reason behind its suppression of showing full potency is the dramatic changes in climatic conditions and low toxic producing ability which could remove deleterious microorganisms in the soil.
Nematode inoculated plant showed the most stunted growth as root knot nematode tends to make effect tomato thereby causing yellowing and stunting of tomato plant and reduce its growth to minimum level (Muhammad Zameer Kayani, 2017).
The main bio-controlled plant was inoculated with nematode and the bacteria. Bio control agent which is *Pseudomonas* interferes with the nematodal activity and suppress its devastating effect on tomato plant either because of toxins present in it. Enzymes present in *Pseudomonas* are proteases and chitinases. Proteases basically interferes with nematodal activity by deteriorate the eggs of *Meloidogyne incognita*. While chitinases play its role as antagnoistic agent against the root knot nematode *Meloidogyne incognita* which basically destroy chitin which the main component of cell wall of nematode (Abo-Elyousr *et al.*, 2020).
So, closing the discussion in a nutshell the result of our present study shows the maximum growth has been shown in healthy plant while the activity of biocontrol agent against nematode shows that bacterial has suppressed nematode activity to some level without making plant look more stunt. While the plant inoculated with bacteria alone have not shown much height as account of its growth promoting nature. While the plant with only nematode inoculated shows relatively less growth (Roland and Perry, 2006).

***Conclusion*:** Keeping in mind the above results healthy plant showed more growth as compared to the other treated plants. Because healthy plants were grown under optimum conditions, with proper availability of water, and were not inoculated with bacteria or nematode. The plants which were inoculated with both bacteria and nematode for biological control show less growth as compared to healthy in which bacteria show less effect on nematode as a biocontrol agent.

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***Ethical statement*:** This article does not contain any studies with human participants or animal performed by any of the authors.

***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

Code Availability: Not applicable

***Consent to participate*:** All authors are participating in this research study.

***Consent for publication*:** Hafiz Younis Raza contributed in the establishment of the Nursery, transplantation of seedlings, and wrote the manuscript, Muhammad Madni helped in nursery and field activity and formatting guidelines, and Zainab Malik and Ain e Zahra contributed to writing the manuscript.

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